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Review Article

Aromatase Inhibitors / SERDs: Envisaging Roles in the Management of Cervical Cancer

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Abstract

Considering that majority of patients with cervical cancer (CxCa) present with advanced disease, current five-year survival statistics of the disease is low; and chemo-radiation forms the mainstay of treatment. Immune tolerance as a hallmark of established tumors has come to stay; with a consequent renewal of interest in immunotherapy. Additionally, since >90% of CxCa is HPV driven, there is potential in immunotherapy targeting the viral oncoproteins. This could serve to enhance effector responses in the tumour microenvironment (TME), simultaneous to overcoming host immune suppression. Clinical response in oncology has dramatically improved following the use of immune check-point inhibitors in anticancer armamentarium. Considering that only a fraction of CxCa patients appear to benefit from the existing checkpoint agents, there is a need to further efforts towards characterizing newer immunotherapeutic targets in the TME. Contextually, there is substantial experimental, epidemiological and clinical evidence, implicating stromal estrogen in CxCa pathogenesis. Additionally, a lowered risk of developing cervical neoplasia was reported in women with carcinoma breast on anti-estrogen therapy. Also, in *exvivo* experiments, Selective Estrogen Receptor Disruptors (SERDs) were found to inhibit the function of tumor infiltrating regulatory T cells, Myeloid Derived Suppressor Cells and carcinoma associated fibroblasts derived from CxCa. Anti-estrogen therapy could therefore act as a checkpoint inhibitor in CxCa targetting locally produced estrogen in the TME. Here we review the potential immunomodulatory activity of aromatase inhibitors/SERDs which could pave the way for drugs interfering with estrogen signalling, some of which could be repurposed for better management of CxCa.

ABBREVIATIONS

CxCa: Cancer Cervix; HPV: Human Papilloma Virus; SERDs: Selective Estrogen Receptor Disruptors; SERM: Selective Estrogen Receptor Modulator; TME: Tumor Microenvironment; Tregs: Regulatory T cells; CAFs: Carcinoma-associated Fibroblasts; AI: Aromatase Inhibitors; MDSCs: Myeloid-Derived Suppressor Cells; TAMs: Tumor- associated Macrophages; NK cells: Natural Killer Cells; CTLA-4; Cytotoxic T lymphocyte– Associated Antigen-4; PD-L1: Programmed Cell Death Ligand 1; ERα: Estrogen Receptor alpha; GPER: G Protein Coupled Receptor; PDE3A: Phosphodiesterase 3A; STS: Steroid Sulfatase; 17βHSD; 17 beta hydroxysteroid dehydrogenase; ICI: ICI *182,780*; CTLs: Cytotoxic T lymphocytes; RU: RU 58668

INTRODUCTION-CERVICAL CANCER

In developing countries which lack both primary and secondary preventive programmes, cervical cancer (CxCa) continues to be a major cause of morbidity and mortality in women [1,2]. Poor prognosis of CxCa is primarily because majority of the cases present with advanced disease, which requires systemic treatment. Presently, the treatment for CxCa is concurrent platinum-based chemo-radiotherapy [3]. In patients with early stage CxCa (IB-IIB), chemo-radiation has been found to offer a 10-15% increase in survival at 5 years post-treatment compared to radiotherapy alone [4,5]. Survival statistics for the International Federation of Gynecology and Obstetrics (FIGO) stage I patients treated by surgery can be excellent: 5-year survival is 96, 95 and 80–93% in the UK, Germany and the USA, respectively [6]. However, more advanced cancers viz. FIGO stages II, III and IV, which are treated with platinum-based chemo-radiation, have lower 5-year survival rates [6]. Overall survival and prognosis of patients of stage IIIB CxCa in India is about 50% [7,8]. Also, current chemotherapy regimens offer response rates of only 35% to 50%, highlighting the need for bettering treatment strategies [9,10]. Hence there is a need for conceptualizing effective therapeutic vaccines against the disease [11], since this would help prevent about 5 million deaths

that would occur in the next 20 years in women who are already infected with HPV [2].

THE TUMOR MICROENVIRONMENT

Solid tumour tissues can be divided into two distinct poorly-demarcated regions viz. the parenchyma (the tumour bed) and the stroma (the Tumor Microenvironment - TME). The tumor microenvironment is a hypoxic, acidic, and immune/inflammatory cell–enriched milieu that plays a crucial role in tumor development, growth, progression, and therapy resistance. The latter is a pathologically active niche that shapes tumor evolution and response to anticancer therapy including immunotherapy [12-16]. The TME encompasses a diverse spectrum of non-malignant cells viz. endothelial cells of blood and lymphatic vessels, mesenchymal stem cells, cancer-associated fibroblasts (CAFs), pericytes and infiltrating immune cells belonging to both the innate and adaptive arms of the immune system, along with the extracellular matrix and secreted soluble factors [12]. Exhaustive studies on the various components of the TME and their crosstalk with tumor cells have aided development of unique therapeutic strategies for improving outcomes in cancer. The vast landscape of intratumoral immune cells can broadly be divided into two groups. One is the protumorigenic / immunosuppressive populations of cells which play a major role in disrupting the capacity for the immune control of cancers: the major players being regulatory T cells (Tregs), Myeloid derived suppressor cells (MDSCs), the M2 polarized tumor associated macrophages (TAMs) etc. The other group comprising antitumorigenic/ effector cells capable of driving potent anti-tumour responses encompasses natural killer (NK) cells, dendritic cells and effector T cells (e.g. CD8+ Cytotoxic T lymphocytes - CTLs and CD4+Th1 cells, etc.). While both effectors and suppressors colocalize in the microenvironment, the immune scale in established cancers gets tilted towards immunoevasion [13]. Besides immunosuppressive cells, immunosuppressive co-inhibitory receptors like cytotoxic T lymphocyte–associated antigen-4 (CTLA-4), and programmed cell death ligand 1 (PD-L1); and immunosuppressive cytokines like IL10 and TGFβ are also active players in the battlefield [14].

IMMUNE INFILTRATES in CxCa

We had earlier reviewed the literature on immune infiltrates in cervical cancer [17]. Hence in this review, a brief discussion on immune infiltrates in CxCa would be restricted to cover published work only from June 2009 onwards, with an emphasis on Tregs. A large body of data demonstrates that CD4+ FOXP3+ Tregs are a strongly immunosuppressive subset of T cells which are a boon for the maintenance of immunological homeostasis and tolerance in infection, transplantation and autoimmunity but a bane for antitumor immunity [18-21]. Natural Tregs (nTregs) are phenotypically CD4+CD25hiCD127lo and express the lineage specific transcription factor FOXP3 - a master regulator which presides over the development, differentiation, maintenance and function of Tregs [22]. Increased frequencies of Treg cells in the TME appear to limit effector immune responses within the tumor, thereby preventing tumor control [23]. The TME of squamous cell carcinomas (SCC) of the cervix is marked by an immunoregulatory environment with increased expression of IDO1, immunosuppressive cytokines TGFβ and IL10; increased ratios of Kynurenine:Tryptophan; various types of Tregs – (both FOXP3+ and FOXP3 negative); reduced ratios of CD4+/FOXP3+ cells and CD8+/FOXP3+ cells; low ratios of M1/M2 subsets of TAMs, Myeloid Derived Suppressor Cells (MDSCs) and anergic Langerhans cells [17,24-31]. Furthermore, such an immunosuppressive TME represented by high numbers of Tregs and PD-L1 expressing TAMs were found to permeate into metastatic lymph nodes (LNs), forming an immune-suppressive cordon around the tumour cells, enabling metastatic spread thereby resulted in a poor disease-free survival [32,33]. Additionally, *exvivo* experiments in our laboratory indicated that under the influence of the secretome of cervical CAFs (C-CAFs) naïve T cells differentiated into Tregs, while their differentiation into Th1, Th2 and Th17 subsets was inhibited (unpublished observation). Hence suppressing Treg cells in cancer immunotherapy is crucial for better prognosis. Additionally, considering the intrinsic antigenicity of CxCa, the prominence of Tregs as a therapeutic target is indisputable. Research studies have indicated that chemotherapeutic agents like cyclophosphamide exhibit anti-Treg properties and hence overall has an improved anticancer effect [34].

The advent of checkpoint inhibitors has heralded a rejuvenation of the field of anti-cancer immunotherapy since the latter boosts the ability of the immune system to recognize and destroy cancer cells, thus remarkably improving the prognosis of patients. Immune checkpoint inhibitor therapies targeting CTLA-4 and PD-1 / PD-L1 enhance antitumor immunity by depleting CD4+ FOXP3+ Tregs and reducing their expansion in the TME. This may be one of the many other mechanisms that favour development of anti tumor immune responses [14,35-37]. One of the earliest targets used for decreasing intratumoral Tregs was CD25 – the IL2 receptor, a constitutive marker on many Treg subtypes [30]. In this model of CxCa, animals receiving anti-CD25 monoclonal antibody before receipt of the E7/Hsp70 DNA vaccine had higher numbers of E7 specific CD8+ T cells and more efficient control of tumors [30]. A resultant proliferation of CD8+ T cells, cytokine production and increase in the ratio of CD8+/Tregs has been recorded. These observations supported by results from several preclinical and early phase clinical studies formed the basis of Treg cell–targeted cancer immunotherapy [38,39]. Ipilimumab and tremelimumab, are two anti-CTLA4 antibodies, the former was the first to be approved by the FDA for the treatment of metastatic melanoma [40]. Likewise, Pembrolizumab and Nivolumab are monoclonal antibodies against PD-1 [41-43]. Currently Phase I/II clinical trials evaluating effects of anti-PD-1 therapy in CxCa are in progress [44-47]. Hence, based on the evidence that HPV positivity correlated with increased PD-L1 expression, checkpoint inhibitors are considered as a second line of treatment in cervical cancer [48]. Pembrolizumab has been approved by the FDA during or after chemotherapy for patients with recurrent or metastatic CxCa with progressive disease [48].

Other immune checkpoint targets under clinical trials are LAG-3, TIM-3 and CCR4 [44-45,49-52]. Antibodies to TIM 3 have an added advantage in anticancer therapy

– they specifically target tumor infiltrating Treg cells or tumor-associated dendritic cells with little action on extratumoral Tregs [42,43,47,49,53]. However, the response

rates with existing check-point inhibitors which are majorly based on data from patients with metastatic disease [48], are low: only 20-30%; reflecting a need to undertake clinical studies on patients with early disease and explore novel targets for reversal of immunosuppression in the TME of CxCa.

ESTROGEN AND CANCER CERVIX

The uterine cervix is an estrogen (E2) responsive part of the female reproductive tract, the epithelium of which proliferates and differentiates physiologically under the influence of hormones during the menstrual cycle. Estradiol has been reported to use both the genomic and nongenomic pathways to signal a cell - by interacting with various receptors viz. Estrogen Receptor alpha (ERα), ERβ, membrane ERα, G protein coupled receptor (GPER), etc. In the canonical pathway, subsequent to binding to ERs, ligand- receptor complexes get translocated to the nucleus and ER interacts with estrogen responsive elements (ERE), on the DNA, following which epigenetic modifications and downstream gene activation ensues. The receptor thus functions as a transcription factor which regulates the expression of genes involved in cell survival and proliferation. The net outcome of these series of events is physiological alterations across various estrogen responsive tissues [54]. The non-genomic action is due to molecular crosstalk with growth factor pathways e.g. insulin growth factor, epidermal growth factor and fibroblast growth factor. Besides having a direct growth promoting action, in some tumors E2 is also pro-tumorigenic by virtue of its capability to modulate various cells of the TME [55].

HPV has long been recognized to be required but not sufficient to cause CxCa [56]. Of the various co-factors incriminated in cervical carcinogenesis E2 has been hypothesized to play a prominent role. Firstly, a series of pooled analysis of several case control studies have indicated that long-term use of exogenous estrogens in the form of hormonal contraceptives is associated with an increased risk of CxCa [57-61]. Likewise, several studies conducted in different continents indicated that exposure to endogenous estrogens by way of multiparity too increases the risk of squamous cell carcinoma of the cervix in HPV-infected women [57,60,62-63]. Various hypotheses explaining the observed relationship between estrogens, HPV and CxCa have been succinctly reviewed earlier [58]. While a marginal association between use of combined oral contraceptives (COC) and risk of acquisition of new HPV infections has been observed, evidence on COC likely promoting HPV persistence remain divided [58]. While physiological concentrations of E2 has been shown to induce expression of HPV oncogenes in cervical cancer cell lines [64-67], and promote the proliferation and inhibit apoptosis of cancer cells [58]; the phenomenon appears to be dose dependent – with high concentrations of the hormone as seen during pregnancy, stopping translation to promote apoptosis [68,69]. Secondly, both E2 and ERα were proven to be necessary to induce CxCa in the *K14HPV16* transgenic mouse model of cervical carcinogenesis; and as a corollary Selective Estrogen Receptor Modulators (SERMs), were effective in controlling the development of cancer [70-74]. Thirdly, in an HPV18 transgenic mouse model, E2 was found to induce increased expression of HPV E6 and E7 oncogenes, - major driving force for cervical carcinogenesis [75]. Paradoxically though, in human disease, epithelial ERα expression, consistently declines with disease progression through cervical intraepithelial neoplasia (CIN) to invasive CxCa - both at the RNA and protein levels [76-82].

Earlier reviews incriminating E2 and ERα in cervical carcinogenesis assisted by HPV oncogenes have recommended the use of SERMs for treating the disease, arguing both for [83], and against the proposition [84]. The pro-apoptotic capability of non- physiological concentrations of E2 on CxCa cell line - HeLa was recently demonstrated [69]. Around the same time, using various receptor antagonists, another elegant study uncovered that in HeLa cells, E2 brought about apoptosis in a non-ER/non GPER fashion by interacting with phosphodiesterase 3A (PDE3A) - infact the latter has gained the distinction of being named as a new ER [68,85]. In this article, we review the role of E2/ERα in potentiating stromal cells and infiltrating immunosuppressive immune cells in cervical carcinogenesis. We earnestly hope that the article would provide food for thought for considering the use of antiestrogen therapy in the management of CxCa.

The stroma in the normal cervix has been shown to express ERα in as high as 93.7% [77], which is independent of changes in plasma hormonal concentrations seen during the menstrual cycle, perhaps indicating local synthesis [77,79,81,86]. Studies done in our laboratory showed that the microenvironment of CxCa was rich in the hormone E2, even in the absence of raised plasma levels (Figure 1A)- the distribution being mainly intracytoplasmic in the tumor epithelial cells and both intracytoplasmic and intranuclear in the stromal and infiltrating immune cells (Figure 1B) [87,88]. The enzyme aromatase too had a parallel distribution in the CxCa tissues thereby indicating that the hormone was being synthesized locally within the tumors (Figure 1B) [88,89]. In addition to aromatization of substrates, one needs to bear in mind that there are other alternative pathways of E2 synthesis in the tissues viz. from estrone sulfate (E1S), through Estrone (E1) by the actions of steroid sulfatase (STS), and 17 beta hydroxysteroid dehydrogenase (17β HSD) (Figure 2). This alternative pathway has been demonstrated in the cervical cancer cell line HeLa [90,91], although their role in the local synthesis of the hormone in clinical cases of CxCa still remains to be demonstrated.

About 30 to >50% of stromal cells express ERα in CIN and invasive squamous carcinomas of the uterine cervix. The distribution of the receptor in the stroma was uneven across the tumours, being independent of the stage of the disease and was surrounded by ERα-negative tumour cells [79,86-88,92]. Both fibroblasts and subsets of lymphocytes were amongst the cell types expressing the hormone receptor [87,88]. Raloxifene and fulvestrant (ICI *182,780* - ICI), are ER antagonists used in the treatment/prevention of human breast cancer and are classified under the category of SERMs and Selective Estrogen Receptor Disruptors (SERDs), respectively. Both the drugs efficiently cleared cancer and its precursor lesions in both cervix and vagina in *K14E6/K14E7* double transgenic mice [83]. A subsequent *posthoc* analysis on the topic, however, ruled out the possibility of long term SERM treatment being useful in the prevention of *Carcinoma In Situ* (*CIS*) and CxCa in humans arguing that animal experiments could not be extrapolated to humans [84]. The objection may perhaps have been justified then, since in the mouse

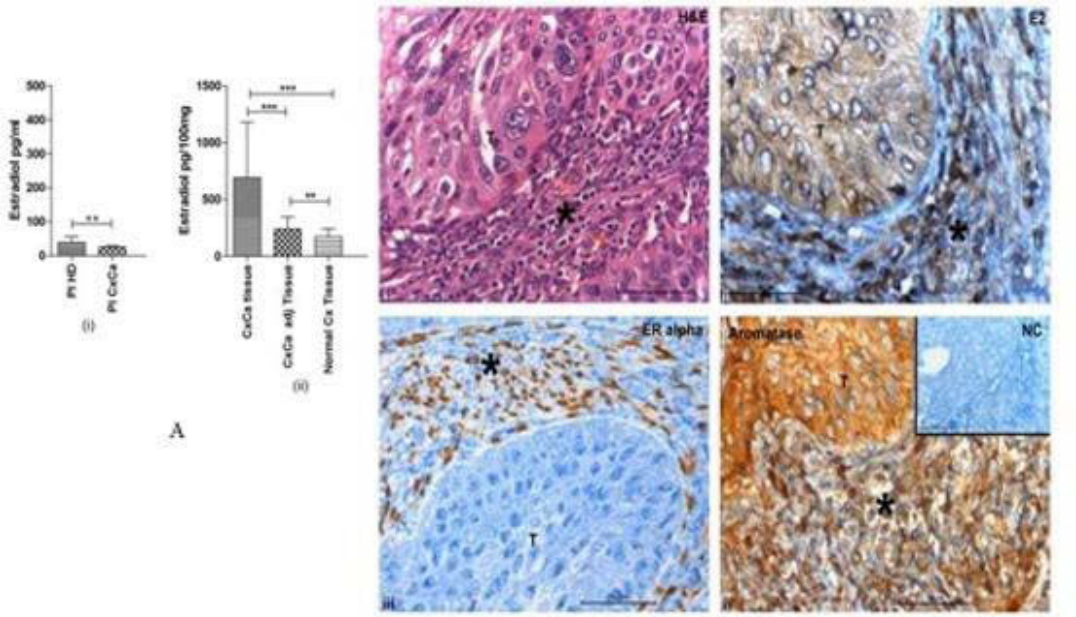

Figure 1 Cervical tumours are enriched in oestradiol (E2) and express oestrogen receptor α..

Figure 1A: (i) Concentrations of 17β-oestradiol as determined by ELISA in blood plasma from healthy donors (Pl HD) or patients with CxCa (Pl CxCa) as well as in (ii) tissue samples of cervical tumours (CxCa), areas adjacent to the tumours (CxCa adj), and healthy cervices (Normal Cx). Graph shows mean values ± SEM of n=30 per group.

Figure 1B. Staining distribution of 17β oestradiol, oestrogen receptor α, and aromatase in a representative tissue section of SCC cervix. Upper left image (i) shows haematoxylin and eosin staining of a tumour section; upper right image (ii) shows E2 staining which was predominantly cytoplasmic in the tumor and both nuclear and cytoplasmic in the stroma and infiltrating cells; lower left image (iii) shows the nuclear staining of ERα in the stromal cells only; lower right image (iv) shows aromatase expression detected in the cytoplasm of the tumour, stroma and infiltrating cells. Inset: normal rabbit serum negative control. Symbol T indicates tumour location in each picture; * indicates stroma. Images are representative of n=30. (Adurthi et al, Sci Rep. 2017 Dec 11;7(1):17289. doi: 10.1038/s41598-017- 17102-w.)

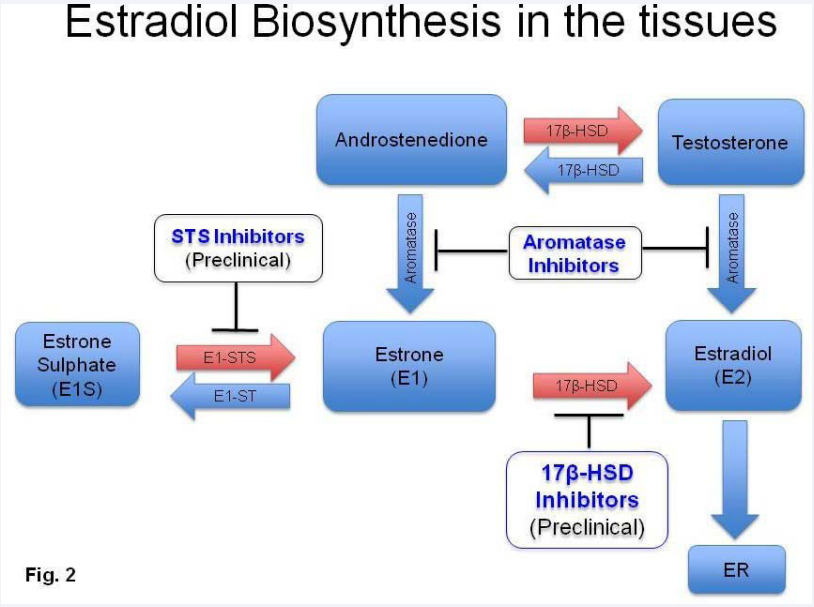

Figure 2 Diagrammatic representation of biosythesis of Estradiol in the peripheral tissues.

High levels of circulatory E1S (Estrone sulfate) has been reported especialy in post menopausal women. Estrone sulfatase (STS) converts E1S into estrone (E1) in the peripheral tissues, which subsequently gets reduced to estradiol (E2) by type I 17β- Hydroxysteroid Dehydrogenase (17, β-HSD). Estradiol in the tissues binds to intracytoplasmic or membrane Estrogen Receptors (ER) and carries out its function. Development of STS inhibitors and 17, β-HSD inhibitors are in the preclinical stage. E1 - Estrone; E2 – Estradiol; E1S: Estrone sulphate; E1 – STS Estrone sulfatase; E1 ST (Estrone sulfotransferase).

model but not in human CxCa, the tumor epithelium retained the expression of ERα. A recent review has discussed both the pro and anticancer effects of the steroid hormone in HPV positive cancers majorly focussing on the tumor epithelium, with minimal reference to the stroma and the immune infiltrates [93]. In an interesting observation, Ormeloxifine an oral contraceptive and a SERM have been advocated as a promising treatment for CxCa. The drug was demonstrated to block multiple signaling pathways particularly PI3k and Akt, inducing apoptosis of CxCa cell lines both in *in vitro* experiments and in an orthotopic animal model [94]. It would indeed be interesting to investigate the action of this SERM on tumor infiltrating immune cells considering that, this is also one of the pathways activated by E2 in Tregs amongst the many others [95].

The tumor epithelium however, cannot be viewed in isolation. A dynamic bidirectional cross talk between the three components viz. the HPV infected epithelium, the stroma and immune infiltrating cells plays a major role during carcinogenesis [71,96]. Drawing from their earlier work and using a sophisticated approach of stromal specific deletion of ERα, Lambert's group proved that E2 signaling in the stromal cells drives tumorigenesis in the epithelium through paracrine mechanisms [82,97]. On similar lines, studies on gene expression profiling of *ex-vivo* cultured CxCa Associated Fibroblasts (C- CAFs), showed CAFs to express *ERα* and support tumor growth by promoting epithelial cell migration, proliferation, angiogenesis, metabolism, epithelial-to-mesenchymal transition and inflammation [87]. Further, using two categories of ER antagonists viz. a SERM and a SERD, modulation of genes associated with cell cycle and metabolism, affecting angiogenesis and cancer progression, was seen, proving thereby that the ERα signalling partly controlled the function of C-CAF [87]. HPV-dependent, E2-induced stromal genes are envisaged to be microenvironmental factors critical for cervical carcinogenesis [68,87]. Also, there is now growing evidence that molecular crosstalk between various components of the TME like CAFs and infiltrating immune cells can influence antitumor immunity [16]. Interestingly, as a consequence of all this research, stromal ERα singaling has been suggested as a therapeutic target for CxCa [98].

ESTROGEN IN INFILTRATING IMMUNE CELLS in CxCa

In addition to the direct carcinogenic action of E2 in CxCa, an indirect action of the sex steroid hormone, is induction of anti-inflammatory and regulatory immune responses which could potentiate HPV mediated cervical carcinogenesis [99]. Estradiol, is a powerful immunosuppressor and acts by: (i) recruiting MDSCs from the bone marrow into the spleen and tumor beds [100] (ii) augments the immunosuppressive activity of granulocytic MDSCs; via ERα [100] (iii) drives and polarizes infiltration of tumors with M2 type TAMs, (iv) promotes VEGF expression in them thus further enabling M2 recruitment; (v) increases the expression of granzyme B inhibitor - proteinase inhibitor-9 which counters the action of granzyme B mediated killing by NK cells and CTLs [55]. Pioneering studies in mice have proven that E2 administration expanded Tregs and induced overexpression of the transcription factor Foxp3 – a signature molecule of Tregs [101]. Additionally, a positive feedback loop between the hormone and infiltration of certain tumors by M2 type TAMs has been reported [55].

Cervical tumors harbour E2 [86-88], and Tregs (intra tumoral, draining LNs or circulating), had the highest intracellular levels of the hormone [88]. Estrogen is one of the various factors which regulate the functions of Tregs [102]. Probable mechanisms of action of estrogens could be modulation by signalling via ERα/GPER [88,101,103-108]. Hence manipulation of E2 action using ER disruptors or modulators may unravel novel approaches to treat CxCa.

SERMS VS. SERDS IN THE MANAGEMENT CERVICAL CANCER

In *ex-vivo* experiments on tumor infiltrating and circulating Tregs, while SERDs (ICI and RU 58668), totally inhibited expression of both ERα and FOXP3 [88], MPP - a SERM, downregualted *ERα*, but failed to alter expression of *FOXP3* (unpublished observation). Although the two SERDs – ICI and RU are chemically distinct, upon binding with ERα, the conformation of the receptor may be altered such that a similar protein – protein interaction surface is presented in both instances. Eventually, this may be flagging off the degradation of the ER-ligand complex by the proteasomal pathway [109]. Considering that (i). the conformation or shape of ER is determined by the chemical structure of the ligand which binds to it (ii) this binding directly affects the nuclear fate and protein turnover of ERα independently of its impact on transcription (iii) changes induced in the receptor upon binding to the ligand is ligand-specific (iv) this conformation is very crucial since it impacts protien – protein interactions and hence the ability of the receptor to interact with coregulatory proteins (i.e. coactivators and corepressors) (v). which eventually is critical to the regulation of target gene transcription (vi). locus specific variation in the engagement of coregulators has been observed for ERα. Hence it is quite likely that different ligands regulate engagement of specific ERα coregulators at the promoter of *FOXP3* gene, which ultimately crucially affects the transcription of FOXP3 and hence the function of Tregs. Also, the relative expression of coactivators and corepressors, the subtype of ER, and its target gene promoter are known to affect the biocharacter of SERMs [110,111].

Therefore, considering the results of the effect of ICI on CAFs [87], Tregs [88], MDSCs [100], evidence of the efficacy of ICI in the treatment of CxCa in a mouse model [74], and our unpublished observation with SERM being ineffective in downregulating *FOXP3*, we opine that there is a strong case for undertaking clinical trials on the efficacy of SERDs in the management of CxCa. The crucial role played by the E2 pathway in the TME of various solid tumors has recently been highlighted [55]. Additional spinoffs of the use of ICI could be assisting the cytotoxic and chemo-radio-sensitization actions of cisplatin [112,113].

SERDs (ICI and RU), majorly act through the genomic pathway of ER signaling in cancer cells to inhibit gene function [114]. We observed that intratumoral Treg cells also showed a direct influence of SERDs (ICI and RU) on the canonical ER signalling pathway - thus inhibiting FOXP3 expression and Treg cell function [88]. However, how and to what extent E2 influences the FOXP3 negative population of CxCa infiltrating Tregs still needs

to be explored [25,115]. Additionally, ICI was also seen to inhibit the immunosuppressive function of granulocytic MDSCs in *exvivo* studies and orthotopic animal model of MDSCs in CxCa [100].

We also found ERα expression amongst infiltrating immune effector cells like CD4+ effectors, CD8+ CTLs in the TME although to a lower extent when compared to Tregs [88]. Hence while the action of ICI on other infiltrating immune cells also needs to be investigated, in all probability, limiting the availability of E2 in the TME would also help energize other subsets like Th1[103,116] and NK cells [117]. A positive flip side of the action of anti-estrogens is that they may also help regain granzyme B expression in HPV16-E7 expressing keratinocytes [118].

AROMATASE INHIBITORS IN THE MANAGEMENT OF CxCa

Arresting intratumoral E2 production with the use of aromatase inhibitors (AI), is a logical alternative to the use of SERDs, since in Tregs, E2 has also been shown to act non-genomically through membrane ER/GPER [95,106]. This was corroborated by our *exvivo* observation: E2 supplementation of ICI treated tumor infiltrating Tregs, partially reversed their suppressive function [88]. Considering that E2 influences Treg suppression by affecting multiple regulatory elements, both PD1 dependent, PD1 independent, FOXP3 dependent and FOXP3 independent pathways [101,103,104], blocking local production of E2 may be a more effective therapeutic option in the management of CxCa than blocking of ERs by way of using SERMs or SERDs. In support of the use of AI, is a recent population based study in breast cancer patients proving that long-term anti- estrogen use, including AI could reduce the incidence of cervical neoplasia [119]. Classically, protection offered against occurrence of high grade cervical dysplasia was seen only in women over the age of 50 years – which once again emphasizes local production as the main source of E2 in the tissues [91]. While Anastrozole has been shown to suppress the differentiation of naïve T cells into Tregs, increase IFNγ, IL12, and reduce IL4, IL10 in animals, Letrozole has been proven to be effective in reducing circulating Tregs in carcinoma breast patients responding to treatment [120,121]. While considering the use of AI, for targeting intratumoral synthesis of E2, one needs to consider other possible pathways of generation of E2 within the tumor tissues viz. from E1 - which is considered a major source of E2 in postmenopausal women [91]. This becomes relevant for designing therapeutics against the actions of 17–β HSD and STS (Figure 2) [122,123]. Also pertinent is the need to study common genetic polymorphism in CYP19A1 and ESR1 genes which would determine the variation in response to AIs and SERDs respectively.

HPV THERAPEUTIC VACCINES

Cervical cancer is etiologically linked to persistent infection with high-risk HPV genotypes [124]. Therefore various immune based therapies including those targeting the viral oncogenes E6 and E7 have shown promising results in clinical trials in a spectrum of HPV-associated disease [11,125-128]. Agents that modulate the TME have an added advantage of being able to increase the efficacy of therapeutic vaccines [2]. There are a number of ways by which Tregs could be targeted viz.: by reducing their number, inhibiting their function, curtailing their influx into the TME and suppressing their generation in the periphery. Hence, we envisage that combining anti estrogen treatment with HPV therapeutic vaccines would be that magic bullet which would serve to be a three-pronged attack. Firstly, it would aid in reversing the immunosuppression in CxCa by inhibiting the suppressive function of intratumoral Tregs and MDSCs. Secondly, the function of CAFs in the tumor milieu would be simultaneously altered. Consequently, the specific immune response to the vaccines could be enhanced, thus promoting better immune control of tumors. Thirdly, AI use would perhaps check intratumoral E2 production which would perhaps also counter nongenomic signaling in cancer cells too.

We are prompted to classify antiestrogen therapy as a check-point inhibitor especially so, for CxCa. Whether it deserves the right to be called a universal check-point inhibitor for other tumors as well remains to be seen.

DISCUSSION & CONCLUSION

The steroid hormone E2 is a well-established pro-tumorigenic agent primarily by its direct genomic/non-genomic action on tumor cells. Estrogen has been considered a co- carcinogen, at least in the early stages of HPV mediated cervical carcinogenesis. Local production of E2 in CxCa tissues is partly through elaboration of aromatase. We have put forth this perspective on E2 being a crucial player in regulating the intratumoral immune response in CxCa by controlling the functions of CAFs, MDSCs and Tregs. Combining HPV oncoproteins with AI/SERD based immunotherapy and/or chemotherapy, on similar lines as of combination with chemotherapy/checkpoint inhibitors may be effective to counter Treg and MDSC mediated immunosuppression and help arrest growth of CAFs within tumors which would thereby improve the prognosis of CxCa [72,74,97,121,129-131]. Anti E2 treatment could thus become a new group of check-point blockade drugs functioning to antagonize intratumoral immunosuppression in CxCa. The need for clinical trials to evaluate the efficacy of AI and SERDs in the management of CxCa hence appears justified. As a prelude to clinical trials, comprehensive characterization of expression of ERα, aromatase, 17β-HSD, STS in the CxCa TME and their gene polymorphisms in large cohorts of patients across continents is warranted. In depth studies to understand the translational relevance of the proposed inhibitors is the need of the hour e.g. post treatment 19 evaluation of pharmacodynamic changes including those in the tumor immune infiltrate and associated clinical changes.

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